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U. S. ARMY MEDICAL RESEARCH AND NUTRITION LABORATORY  
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Denver, Colorado 80240

1 August 1963

SUMMARY OF SIGNIFICANT FINDINGS BY SGO CONTRACTORS

For Reporting Period: September 1962 - March 1963

This report covers the reports of the following contractors: Armed Forces Institute of Pathology, Blood (Vanderbilt), Bone (Oregon State), Calandra (Industrial Bio-Test Laboratories), Clarkson (Wake Forest), Deichmann (Miami), Deisy (St. Louis), Johnson (Univ. of Illinois), Loosli (Cornell), Meneely (Vanderbilt), Monsen (Univ. of Illinois), Reber (Univ. of Illinois), Tinsley (Oregon State), and Watson (VPI).

A. Progress Reports. Wholesomeness Tests.

LT. COL. ROSS (AFIP): Essentially all expected material has been received (2,990 rats, 297 dogs and 39 monkeys) and reviewed. Statistical tabulation is in progress. Tentative conclusions: Statistical analyses have shown that histopathologic lesions are not consistently associated with irradiation levels of food ingested. Sex differences have been more prevalent than differences between feeding groups.

Thyroiditis in dogs. A special study was made of this lesion by AFIP in 251 dogs from 9 contractors. This lesion was found in 15% of the animals. With the exception of a sex difference at the 5.58 Mrad level, males having a greater incidence, no significant differences could be found which could be attributed to an irradiated food or level of irradiation.

CLARKSON: Dogs - beef (reproduction study). Electron irradiated (5.58 Mrads) or nonirradiated beef was incorporated into commercial dog meal as 35% dry solids and fed to each of two groups of litter mates (3 males and 15 females per group). Weekly supplements of non-irradiated vitamins A, D and E were administered. There were no significant differences between the two groups in hematologic data, gestation periods, pup body weights at birth or weaning, number of estrus periods, conceptions, conceptual failures, number of pups whelped or weaned. There was a highly significant difference between the two groups in the age in days to first estrus. Test group  $322.8 \pm 46.4$  days and control group  $454.8 \pm 94.0$  days. No reason for this difference could be given.



LOOSLI: Dogs - beef (reproduction study). See Clarkson for diet and number of animals per group. Proven male beagles were used in mating. No important differences have been noted in reproductive performance or growth between the irradiated and nonirradiated beef groups. No important differences have appeared in days of age to first estrus (see Clarkson).

MONSEN: Mice (heart lesion). Recent feeding tests with irradiated or nonirradiated evaporated milk, cooked or uncooked, with or without vitamins have shown that the incidence of heart lesions was least with nonirradiated, uncooked, with vitamins (23%) and highest with nonirradiated, cooked, without vitamins (91%). The incidence of heart lesions with irradiated, cooked or uncooked, with vitamins was 80 and 75%, respectively. While the causative agent in the Cb strain is not evident, it appears that cooking has the same effect as irradiation on evaporated milk. Vitamin supplementation is beneficial in almost every instance.

REBER: In reviewing past data of animals fed irradiated beef, it was noted that the groups fed irradiated beef were consistently (but not statistically significant) inferior in growth rate to the nonirradiated beef groups. It was also noted that there seemed to be a consistently greater difference between the two levels of irradiation (2.79 and 5.58 Mrads) than between the high level of irradiation or control. These data are being verified; however, it does not seem that there is sufficient alteration of the meat protein to account for the observed growth differences.

Methionine showed its protective effect on prothrombin levels in rats fed irradiated beef even when high levels of vitamin A were fed.

TINSLEY: Rats - carrots. It was previously reported that rats fed irradiated carrots, stored at room temperature for at least 6 months, had significantly decreased growth rates and decreased liver vitamin A stores when compared to rats fed nonirradiated carrots which were stored frozen. The decreased growth and liver vitamin A stores were not found in rats fed irradiated carrots which were stored at 0° F. Apparently, storage temperature is more a factor than is irradiation.

No conclusive evidence has been found to relate increased liver cytochrome oxidase and oleate/arachidonate ratio of liver mitochondrial fatty acids in rats fed irradiated pork.

WATSON: Dogs - shrimp. Pure-bred beagles were found to have an increased incidence of thyroiditis when fed irradiated shrimp which had been stored for 3-5 years. Post-mortem examination of pound dogs (40 animals to date) showed that 30% had mild microscopic lesions of the thyroid. Both of these studies are being continued. See Ross, AFIP).

Rats - shrimp. Feeding studies with rats have not produced productive data and have been discontinued.

B. Final Reports. Wholesomeness Tests.

BLOOD: Section 2, Final, Histopathology.

This final report contains the histopathologic data of the long-term feeding studies previously reported by Kline et al. as Final, Section 1, Procedures and Laboratory Results, May 1961. Diets contained 35% (dry solids) irradiated feed (0, 2.79 or 5.58 Mrads) added to commercial animal meal. Oranges were irradiated to 150 or 300 Kreps and were incorporated at the 25% level into commercial meal. Dog and Rat diets were supplemented with vitamins A, B and E.

1. Dogs - Chicken, beef or jam. Breeding performance was poor regardless of treatment or diet.
2. Rats - Beef. The hemorrhagic syndrome was not observed.
3. Monkey - Peaches, whole or peeled oranges. Monkeys on peach diet developed scurvy-like symptoms correctable by ascorbic acid. Whole orange diet did not produce increased intraocular pressure.

Based on hematologic values, growth, reproduction, lactation, food consumption, feed efficiency, urine analysis and histopathologic examination, it was concluded that there was no indication that inclusion of irradiated feeds in the diets of dogs, rats or monkeys was harmful.

BONE: Final.

This is a final report on the histopathologic studies of the previously submitted final report by I. J. Tinsley et al., The Growth, Breeding, Longevity and Histopathology of Rats Fed Irradiated or Control Foods, Oregon State University, September 1961 (DA-49-007-MD-580). Pork, jam, carrots and peaches, each irradiated to 2.79 and 5.58 Mrads, and flour irradiated to 37 and 74 Krad were each fed to rats as 35% dry solids in a semipurified diet. It was concluded that the irradiation preserved foods used in this study did not produce an increased pathology, carcinogenic potential or uniquely deleterious biologic effect in rats.

CALANDRA: Final.

This is a final report of a mouse carcinogenicity study. Six foods (cod fish, beef stew, chicken stew, green beans and peaches each irradiated to 5.58 Mrads and flour irradiated to 74.4 Krad) were incorporated into a 100% irradiated diet containing 16.67% dry

weight of each feed. Each of two strains of mice (Cal A and C<sub>3</sub>H/NT) were fed the composite diet (irradiated or nonirradiated) for two years. Diets were supplemented with nonirradiated vitamins, minerals and liver concentrate. Attempts to establish colonies of multiparous females were unsuccessful. Growth, mortality and tumor incidence was studied. No significant differences in either strain could be found in the parameters studied which could be attributed to diet or irradiation.

BEICHMANN: Final.

This is a final report of a mouse carcinogenicity study. A 100% irradiated diet was made from 5 foods (tuna fish, beef, corn, sweet potatoes and fruit compote) each of which was irradiated to 5.58 Mrads. Four strains of mice (C<sub>3</sub>H, C<sub>57</sub> black, DBA and Swiss) were fed the irradiated or nonirradiated composite diet for periods of up to 26 months to a total of 2834 mice. Diets were supplemented with nonirradiated vitamins. It was concluded that there were no meaningful differences in body weights, feed consumed, mean survival or tumor incidence between the irradiated or nonirradiated diet groups. Irradiation of the foods used does not render them carcinogenic for mice.

MEENEELY: Final.

Canned pork, beef, ham, chicken and bacon were irradiated with Co-60 or electron beams of 8, 11, 12, 13, 14 or 16 Mev to 5 Mrads. Thermally processed nonirradiated foods from identical food lots were used as background controls. No positive evidence of induced radioactivity was detected in foods irradiated with Co-60 or with electrons below 12 Mev. Above 12 Mev, the isotopes Sc-47, Xe-133m, Mn-54, Sc-46, Na-24 and Sc-48 were detected and identified by energy and half-life measurements.

Electron irradiated foods showed large sample-to-sample variance. Further work is suggested, particularly for food irradiated with electron beams in terms of sample-to-sample variance and to define more precisely the detection limits.

C. Progress Reports. Miscellaneous Studies.

BOISEY: Results of past experiments with extracted and non-extracted irradiated and fresh beef, as well as with other non-irradiated proteins, suggest that the hemorrhagic syndrome may be caused by destruction of minute amounts of substances possessing vitamin K activity. It is estimated that about 0.2 µg of vitamin K<sub>1</sub> equivalent per gram of beef fat is destroyed by irradiation. Fresh beef fat contains less than 1 µg of vitamin K<sub>1</sub> equivalent per gram. The vitamin K<sub>1</sub> equivalent per gram of fat as calculated from the data of Mamasch et al. is 2-3 µg.

JOHNSON: Results of past experiments have shown that the biological and energy value of irradiated foods are comparable to heat processed foods. Beef is a poor source of vitamin K and irradiation destroys most of the vitamin K present without evidence of production of vitamin K antagonists. The primate appears to be far less susceptible to vitamin K deficiency than the rat, pig or chick. It is concluded that, if one considers the variety of foods in a normal human diet, irradiated beef is perfectly safe for human consumption.

Vitamin K does not function in general protein synthesis nor is oxidative phosphorylation in liver mitochondria affected in vitamin K deficiency. Rat liver microsomal prothrombin levels are decreased greatly in vitamin K deficiency.

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